Transactions of the Royal Society of S. Aust. (1993), **117**(4), 179–182.

EFFECT OF *ACROBELOIDES NANUS* (NEMATODA: CEPHALOBIDAE) UPON THE SURVIVAL OF *PSEUDOMONAS CORRUGATA* (EUBACTERIA) IN PASTEURIZED SOIL FROM KAPUNDA, SOUTH AUSTRALIA

by MAARTEN H. RYDER*† & ALAN F. BIRD*

Summary

RYDER, M. H. & BIRD, A. F. (1993) Effect of *Acrobeloides nanus* (Nematoda: Cephalobidae) upon the survival of *Pseudomonas corrugata* (Eubacteria) in pasteurized soil from Kapunda, South Australia. *Trans. R. Soc. S. Aust.* **117**(4), 179–182, 30 November, 1993.

Acrobeloides nanus and *Pseudomonas corrugata* were co-inoculated into the same pasteurized field soil. The population of the nematode increased ten-fold in eight weeks, whereas the population of the bacteria decreased with time, in all cases. After eight weeks at 15 °C, the population of the introduced bacteria was significantly lower where *A. nanus* had been added to the soil. At 22 °C the population of *P. corrugata* was much lower irrespective of the presence or absence of *A. nanus*. The feeding of nematodes on bacteria in soil helps to explain the observed decrease in the population of microbial control agents introduced into the soil in field experiments.

KEY WORDS: *Acrobeloides nanus*, bacteria, biological control, Cephalobidae, nematodes, *Pseudomonas corrugata*, soil, take-all.

Introduction

With increased interest in the use of biological control agents as alternatives to chemical control measures has come an interest in the fate of these biocontrol agents in the soil. In particular, information on the effect that bacterial-feeding nematodes may have on bacterial biocontrol organisms is sparse. Accordingly, we have isolated, cultured and microscopically examined a biocontrol bacterium and a nematode from the same agricultural soil and have studied their relationship both on agar plates and in the soil.

The bacterium *Pseudomonas corrugata* isolate 2140R is a biocontrol agent against the root-pathogenic fungus *Gaeumannomyces graminis* var. *tritici* (Ryder & Rovira 1993). This fungus causes the root disease take-all which is responsible for serious yield losses in cereal crops in southern Australia and elsewhere in the world. *P. corrugata* 2140R is a rifampicin-resistant derivative of the parent strain and is, therefore, easily identified. The nematode which we used was *Acrobeloides nanus* (Anderson 1968; Boström & Gydemo 1983; Nicholas & Stewart 1989; Bird *et al.* 1993), a cosmopolitan, bacterial-feeding, free-living organism that is widespread in agricultural and arid soils throughout Australia. The nematode *A. nanus* can use the bacterium *in vitro* as a nutrient source and is able to complete its life cycle and reproduce while feeding only upon *P. corrugata* (Bird & Ryder 1993). We show that the presence of *A. nanus* can lead to a reduction of bacterial numbers when both organisms are inoculated into pasteurized soil.

* CSIRO Division of Soils and † Cooperative Research Centre for Soil and Land Management, Private Bag 2, Glen Osmond, South Australia 5064, Australia.

Materials and Methods

Nematode

Acrobeloides nanus was isolated, by means of a misting apparatus, from the dry soil, collected six years previously (in 1986), from which *P. corrugata* had originally been isolated (Bird & Ryder 1993).

Bacteria

Pseudomonas corrugata 2140 was isolated originally from the rhizosphere of wheat seedlings that had been grown in a field soil, a red-brown earth pH 6, collected from Wagga Wagga, New South Wales (Ryder & Rovira 1993). The rifampicin-resistant derivative *P. corrugata* 2140R was isolated as a spontaneous mutant growing on Nutrient Agar, by J. Brackin, Monsanto Co., St Louis, USA.

Soil and pasteurization conditions

Field soil, a sodic red-brown earth (Stace *et al.* 1968), more recently defined as a fine mixed thermic calcic natrixeralf (Soil Survey Staff 1990), from Kapunda, South Australia, that had been air-dried and sieved (<3 mm) was used in our experiments. Immediately prior to use, it was steam sterilized at 75 °C for 15 min in order to kill all nematodes but not all bacteria.

Inoculation of soil with bacteria and nematodes

Treatments consisted of nematodes + bacteria (+N+B), nematodes alone (N), bacteria alone (B) and controls without either. Each treatment was replicated five times. The experiments were run at 15 °C and 22 °C and were harvested at eight weeks from commencement.

The nematodes, grown on *P. corrugata* 2140, the parent bacterial strain which is sensitive to

rifampicin, on malt extract agar (Oxoid) were washed from the Petri dishes with sterile distilled water, centrifuged three times in sterile distilled water (200 × g for 10 min) and added as aliquots to the soil samples. In the +N+B treatment, bacteria were added to the suspension of nematodes immediately prior to inoculation of the soil. At the commencement of the experiment, samples (20 g) from all four treatments were placed on a misting nematode-extraction apparatus and the nematodes in the eluate were counted. The +N+B treatment yielded four nematodes per g of dry soil, and +N alone five. No nematodes were found in the soils with added bacteria (+B) or in the controls (C).

Bacteria (*P. corrugata* strain 2140R) were grown on Nutrient Agar (Difco), containing rifampicin (Boehringer) at 100 mg per litre, for two days at 25 °C. The cells were harvested by washing the plate with 4 ml sterile distilled water. The cell density was adjusted to ca. 2×10^7 colony-forming units (cfu) per ml before inoculation of soil. The starting population of *P. corrugata* was taken as the number of rifampicin-resistant bacteria recoverable from the soil 30 min after inoculation. Five replicate samples were processed for dilution plating. The initial bacterial populations were 1.5×10^6 cfu/g dry soil (+B) and 1.17×10^6 cfu/g dry soil (+N+B).

The air-dried soil was wet initially to 12% (w/v) with distilled water. 20 g portions of moist soil were dispensed into sterile containers (plastic-capped, 120 ml capacity). Either nematodes (+N) or bacteria (+B) alone were added to the soil in 1 ml. For addition of both organisms together (+N+B), the suspensions of nematodes and bacteria were mixed and then added to the soil in 1 ml. Sterile distilled water (1 ml) was added to the control treatment. The organisms were mixed into the soil thoroughly using a glass rod that had been wiped with ethanol, but the mixing was done gently to preserve the nematodes. The final water content of the soil was 19.4% (w/w dry soil).

Incubation conditions

The containers with treated soil were incubated in darkness at either 15 °C or 22 °C. They were capped tightly, but once each week the lids were removed for several seconds in a laminar flow cabinet, to allow gas exchange to occur. The incubation period was eight weeks.

Isolation and enumeration of bacteria

Sterile distilled water was added to each container so that the total volume of soil and liquid was 70 ml. The container was shaken gently for 30 sec to allow the soil aggregates to disperse. One ml was taken for analysis of bacterial populations by dilution plating. The selective medium used was Nutrient Agar containing cycloheximide (75 mg/l) and rifampicin (100 mg/l). The droplet plating method (three replicate 10 µl droplets) was used except for the controls (C) where 0.1 ml of the undiluted suspension was spread on the plate. Bacterial colonies were counted after two days at 25 °C.

Isolation and counting of nematodes

The nematodes in the remaining 69 ml of soil suspension were isolated by means of Seinhorst's two flask technique followed by sieving and counting (Hooper 1986).

Statistics

Populations of nematodes and bacteria were analysed by an analysis of variance of log-transformed data (Genstat Version 5).

Results

The populations of both *A. nanus* and *P. corrugata* after eight weeks in pasteurized Kapunda soil at 15 °C or 22 °C are shown in Figs 1 & 2. There were no significant differences in populations of *A. nanus* at either temperature after eight weeks (bacterial treatment: $F=0.18$, $p=0.68$; temperature: $F=1.29$, $p=0.27$). Where nematodes were added their numbers had increased at least ten-fold since the commencement of the experiment. Addition of *A. nanus* significantly

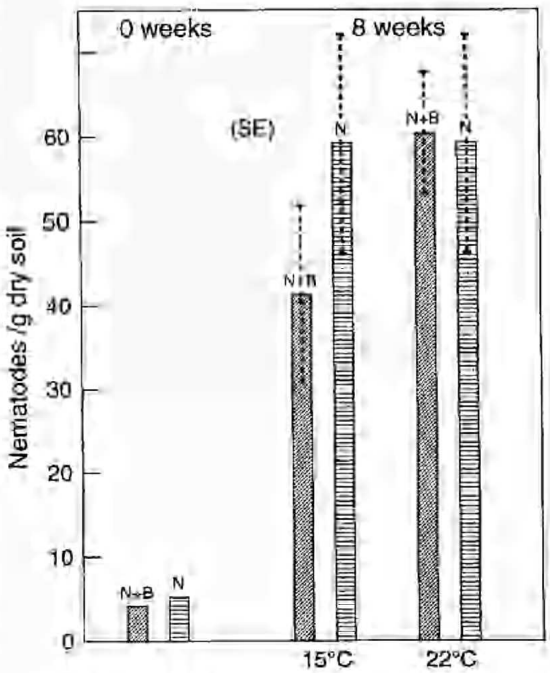

Fig. 1. Histogram showing no significant difference in numbers of nematodes at either temperature after eight weeks irrespective of whether bacteria were added or not. Error bars represent standard error (SE) of the mean.

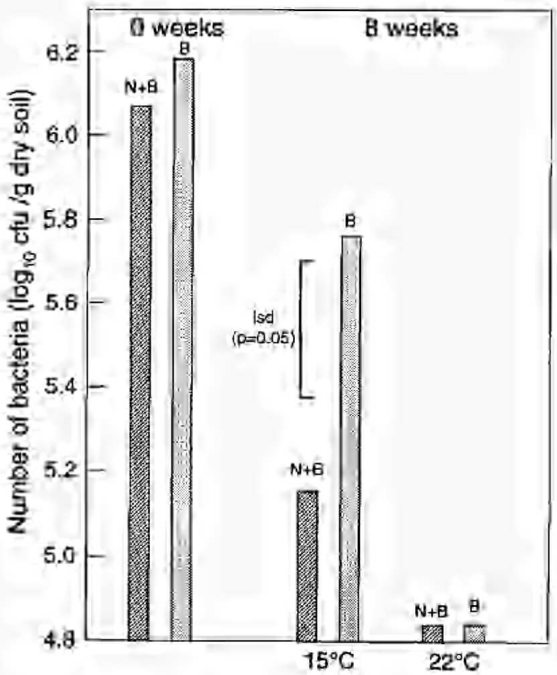

Fig. 2. Histogram showing a significant decrease in bacterial numbers after eight weeks at 15 °C when nematodes were present.

reduced the population of *P. corrugata* over eight weeks at 15 °C (nematode treatment: $F=13.49$, $p<0.001$; temperature: $F=18.53$, $p<0.001$; interaction between nematode treatment and temperature: $F=3.76$, $p=0.038$). Numbers of *P. corrugata* decreased eight-fold in the presence of *A. nanus* compared to two and three quarter-fold in the absence of the nematode. At 22 °C over this period of time, bacterial numbers were reduced, to approximately one twentieth of the population at the start of the experiment, irrespective of whether or not nematodes were present.

Discussion

The presence of *A. nanus* in the soil significantly reduced the population of *P. corrugata* after eight weeks at 15 °C. This temperature closely resembles winter soil temperatures in the field in South Australia, when biological control of *G. graminis* var. *tritici* by *P. corrugata* has been tested. Our results provide a possible explanation for the observed decrease in numbers of pseudomonads in biocontrol experiments conducted in the field (Weller 1983; Ryder *et al.* 1990).

After eight weeks at 22 °C, the numbers of *P. corrugata* were much reduced. This occurred irrespective of whether *A. nanus* was present or not. This suggests that competition with other bacteria, with growth optima close to 22 °C, may be responsible for the decline in numbers of *P. corrugata* in the soil. This decrease in numbers of *P. corrugata* is probably not due to increased temperature because its growth rate *in vitro* is higher at 20-30 °C than at 15 °C (Ross & Ryder, unpubl.).

Pasteurization of the soil resulted in the death of all soil nematodes and many, but not all, of the bacteria. This meant that the *P. corrugata* were introduced into a bacteriologically competitive soil environment. One such competitor, yet to be identified, proved to be a bacterium that formed visible white patches on the surface of the soil. These patches were considerably reduced in the presence of the nematodes and were presumably fed upon by *A. nanus*.

The bacterial-feeding nematode *A. nanus* is both widespread in South Australian agricultural soils and is relatively abundant, forming 12-28% of the total nematode fauna in soil from field plots at Kapunda, SA, sampled in the spring of 1992 (Yeates & Bird in press). This is equivalent to 1-17 nematodes per gram of dry bulk soil. Thus the levels of added *A. nanus* in the experiment reported here, were representative of numbers found naturally in the field.

The ability of *A. nanus*, and other species of bacteria-feeding nematodes, to graze on bacteria may affect the biocontrol performance of introduced bacteria. This may be particularly important for introduced bacteria in the rhizosphere, as there is evidence that bacteria-feeding nematodes can inhabit the rhizosphere in large numbers (Griffiths 1990). The relative populations of bacterivorous nematodes in the rhizosphere and bulk soil should also be of great interest to those who wish to introduce specific bacterial strains into the rhizosphere. Whether the effect of *A. nanus* in decreasing populations of *P. corrugata* can be large enough to decrease biocontrol performance in a natural soil is not yet known.

Interactions between rhizosphere or soil bacteria and plant pathogenic nematodes have been studied by Bookbinder *et al.* (1982). In their study, there were synergistic negative effects when the bacterial strains, including *P. corrugata* isolated from symptomless alfalfa roots, were inoculated together with a range of plant pathogenic nematodes. However, as far as we are aware, ours is the first report of the relationship between a specific soil bacterium and a bacteria-feeding nematode isolated from the same soil. Further studies along these lines with microbivorous nematodes and the bacteria upon which they feed are necessary steps in understanding both the complexity of the interactions between these organisms in the soil environment and the limitations on the success of biocontrol agents in controlling disease. Further research on these lines is also warranted because these nematodes are readily cultured, natural inhabitants of the soil whose inundative release in other situations may prove to be of immense benefit in maintaining soil fertility while reducing the numbers of undesirable microorganisms.

Acknowledgments

The authors wish to thank Ms A. Reid, CSIRO Biometrics Unit, for performing the statistical analyses.

References

ANDERSON, R. V. (1968) Variation in taxonomic characters of a species of *Acrobeloides* (Cobb, 1924) Steiner and Buhrer, 1933. *Can. J. Zool.* **46**, 309-320.

BIRD, A. F., DE LEY, P. & BIRD, J. (1993) Morphology, oviposition and embryogenesis in an Australian population of *Acrobeloides nanus*. *J. Nematol.*, **25**, 606-614.

_____ & RYDER, M. H. (1993) Feeding of the nematode *Acrobeloides nanus* on bacteria. *Ibid.* **25**, 493-499.

BOOKBINDER, M. G., BLOOM, J. R. & LUKEZIC, F. L. (1982) Interactions among selected endoparasitic nematodes and three pseudomonads on alfalfa. *Ibid.* **14**, 105-109.

BOSTRÖM, S. & GYDEMO, R. (1983) Intraspecific variability in *Acrobeloides nanus* (de Man) Anderson (Nematoda, Cephalobidae) and a note on external morphology. *Zool. Scripta* **12**, 245-255.

GRIFFITHS, B. S. (1990) A comparison of microbial-feeding nematodes and protozoa in the rhizosphere of different plants. *Biology and Fertility of Soils* **9**, 83-88.

HOOPER, D. J. (1986) Extraction of free-living stages from soil. pp. 5-30 *In* J.F. Southey (Ed.) "Laboratory Methods for Work with Plant and Soil Nematodes." 6th Edtn (HMSO, London).

NICHOLAS, W. L. & STEWART, A. C. (1989) Experiments on anhydrobiosis in *Acrobeloides nanus* (de Man, 1880) Anderson, 1968 (Nematoda). *Nematologica* **35**, 489-491.

RYDER, M. H., BRISBANE, P. G. & ROVIRA, A. D. (1990) Mechanisms in the biological control of take-all of wheat by rhizosphere bacteria. pp. 123-130 *In* D. Hornby (Ed.), "Biological Control of Soil-borne Plant Pathogens", (CAB International, Wallingford, UK).

_____ & ROVIRA, A. D. (1993) Biological control of take-all of glasshouse-grown wheat using strains of *Pseudomonas corrugata* isolated from wheat field soil. *Soil Biol. and Biochem.* **25**, 311-320.

SOIL SURVEY STAFF (1990) "Keys to Soil Taxonomy" 4th Edtn SMMS technical monograph no. 6 (Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA).

STACE, H. C. T., HUBBLE, G. D., BREWER, R., NORTHCOTE, K. H., SLEEMAN, J. R., MULCAHY, M. J. & HALLSWORTH, E. G. (1968) "A handbook of Australian soils" (Rellim Technical Publications, Glenside, South Australia).

WELLER, D. M. (1983) Colonization of wheat roots by a fluorescent pseudomonad suppressive to take-all. *Phytopathology* **73**, 1548-1553.

YEATES, G. W. & BIRD, A. F. (in press) Some observations on the influence of agricultural practices on the nematode faunae of some South Australian soils. *Fund. and Appl. Nematol.*